IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Skiffington et al.	Examiner:	Beisner, William H.
Reissue Serial No:	10/014,154	Art Unit:	1744
Filed:	December 6, 2001		
Original Patent:	6,180,395		
Original Patent Issue Date:	January 30, 2001		
Title:	Reagent Chamber For Test Apparatus and Test Apparatus		

DECLARATION OF DR. STEVEN J. SAUL UNDER 37 C.F.R. §1.132

I declare:

1. I have been employed in the area of scientific research and development by Charm Sciences, Inc., the assignee of the above-captioned patent application, since 1990. I currently hold the position of Director of Research.

2. I have worked and published extensively in the area of protein chemistry and enzymatic reactions, and I am an expert in this field. In 1983, I was awarded a PhD degree in Biological Science, with an emphasis in enzymology, from the University of Rhode Island, RI. From 1984-1990, I conducted postdoctoral studies in several areas of enzymatic biochemistry, which included the protein biochemistry and reaction mechanisms of several insect systems. Attached, as Exhibit 1 hereto is my curriculum vitae, which lists over 32 publications in this scientific area of which I am an author or a co-author. My curriculum vitae further include seven issued patents in this scientific area of which I am an inventor or co-inventor. Exhibit 1.

3. I have read the Office Action issued in U.S. Serial No. 10/014,154 on September 25, 2004, and I have read provisional patent application 60/001,081, filed July 12, 1995 (hereafter "the '081 provisional").

4. I have extensive experience using and refining techniques for detecting ATP by the luciferin-luciferase enzymatic reaction, and with the use of lysis solutions to extract ATP from cellular samples. I hold three U.S. patents which concern subject matter relating to the use of the luciferase enzyme with various derivatives of luciferin. See, Exhibit 1, page 3.

5. I note that the '081 provisional, e.g., in the second paragraph and in figure 2 describes *inter alia*, "microbialysis [sic] solution and an ATP stabilizer, a buffer optimized for luciferin-luciferase reaction, . . . " "microbial lysis solution," and "buffer optimized for luciferin-luciferase reaction." The meanings of terms such as "microbial lysis solution", "ATP stabilizer", and "buffer optimized for luciferin-luciferase reaction" were conventional and commonly understood by those of ordinary skill in the art in 1995, with such meanings reflected in the literature of the time.

6. By July of 1995, methods and reagents for extracting ATP from cellular samples with lysis solutions were well known and commonplace, and the luciferin-luciferase assay was a well-established and conventional technique for detecting such ATP. In fact, by 1995 it was commonly understood that a main reason for extracting ATP was as a basis for subsequently measuring that ATP by the luciferin-luciferase assay. See, page 14-15 of Stanley, P.E., *Extraction of Adenosine Triphosphate from Microbial and Somatic Cells*, *Methods in Enzymology*, 133:14-22, Academic Press, London 1986) (hereafter "the Stanley review". The Stanley review is attached as Exhibit 2 hereto.

7. Referring to the first paragraph of the '081 provisional, one skilled in the art in July 1995 would have understood the passage "a portable pocket-swab type test kit and method for detecting the presence of ATP on surfaces in water and other biological fluids or foods . . " to refer to a device for detecting a mixed population of cellular contaminants on surfaces and in samples of water, biological fluids, and foods. Stanley, a 1986 review article, points out that cells are commonly present as a mixed population in nonliving

materials such as soil, meat, milk, fruit juices, and clinical samples. Exhibit 2, at page 21.

8. It is my opinion that one skilled in the art in July 1995 would have understood the '081 provisional to have described a reagent composition that included a buffered solution to release adenosine triphosphate (ATP) from the test sample into the solution for subsequent testing by the luciferin-luciferase assay. In fact, it has long been well known and established that ATP release reagents were known to include a buffer composition. For example, the conventional practice of using buffered lysis solutions to extract ATP from cellular samples is reflected in the background section of a published PCT patent application, Andreotti et al., WO 92/20781, November 26, 1992 (hereafter "Andreotti," Exhibit 3 hereto). Andreotti summarizes various then-known ATP releasing reagents, pointing out that ATP releasing reagents were commonly combined with buffers to adjust pH. Exhibit 3, pages 1-3. It was thus well known and established, prior to Andreotti, that ATP release reagents were known to include a buffer composition. Exhibit 3, page 2-3.

9. US Patent No. 5,283,179, Wood, 1994, "Luciferase Assay Method" (hereafter "Wood", Exhibit 4 hereto) provides another example of a buffered lysing solution optimized for extracting ATP and detecting ATP with luciferase enzyme. One solution in Wood consisted of a Tris-phosphate buffer, glycerol or ethylene glycol, Triton X-100, bovine serum albumin (BSA), cyclohexylenediaminetetraacetate (CDTA), and DTT. Exhibit 4, col. 16, lines 10-18. More generally, Wood teaches that typical buffering agents were known to include, e.g., tricine, HEPPS, HEPES, MOPS, Tris, glycylglycine, and a phosphate salt to maintain pH and ionic strength. Exhibit 4, col. 8, lines 14-25.

10. Methods for optimizing buffered solution conditions in order to detect ATP by the luciferin-luciferase assay were well established, conventional, and routine in 1995. Guidance for optimizing conditions for the luciferin-luciferase assay were widely available in the form of review articles in journals that were widely read by those skilled

in the art. One example is Optimization of the Firefly Luciferase Assay for ATP (Webster, J.J., and Leach, F.R., *Jour. Applied Biochemistry*, 2:469-479, Academic Press, London, 1980) (hereafter "Webster", attached as Exhibit 5 hereto). Webster summarizes the relative affect of various buffers on luciferase activity. Exhibit 5., pages 469-470 and Table I.

11. Stanley is a second example of such a review article. Exhibit 2. The Stanley review is a summary of what was already known in the field pertaining to the properties of the ideal extractant, including those properties that were known to have an affect on the ATP-firefly luciferase assay. Exhibit 2, pages 15 and 16. Stanley cites as examples two detergents that were commonly used in 1986: Triton-X-100 and benzalkonium chloride. Considering the four categories of main extractants described by Stanley (Exhibit 2, page 18), one skilled in the art in 1995 would invariably have concluded that a detergent would be the most appropriate for the ATP-firefly luciferase application.

12. A third review article stressing the importance of detergents to the ATP-luciferase reaction is the 1982 review article Effect of Solvents on the Catalytic Activity of Firefly Luciferase (Kricka, L.J. et al., *Archives of Biochemistry and Biophysics*, 217(2), Academic Press, London 1982) (hereafter "Kricka," Exhibit 6 hereto). Kricka describes how a variety of detergents stimulate and/or inhibit the ATP-luciferase reaction. Exhibit 6, page 676.

13. One skilled in the art in July 1995 would have known a detergent to be a necessary component of a lysis solution for releasing ATP into solution in preparation for testing with luciferin-luciferase. For example, U.S. Patent No. 5,004,684, Simpson et al, 1991, "Method for ATP Extraction" (hereafter "Simpson", Exhibit 7 hereto) demonstrates the understanding of those skilled in the art of the importance of using detergent as an extractant. In particular, Simpson uses a detergent-containing buffered solution to release ATP into the test solution for testing, making multiple references to extracting ATP from microorganisms using detergents in buffers that had been optimized

for the luciferin-luciferase assay. Simpson, col. 3-4. In fact, Simpson refers to the Stanley review when citing certain disadvantages to using acids and organic solvents (Simpson, col. 2, lines 49-55). Thus, by 1995 one skilled in the art would have understood that ample guidance was available for determining which detergents and in what concentrations.

14. Further illustration of the use of detergents as lysing agents when the '081 provisional was filed is shown by the summary of the state of the art by Andreotti, and by the patents of Wood and Kolehmainen et al. (Exhibits 3, 4, and 8). Wood referred to the use of detergents or surfactants as being routine. Exhibit 4, col. 8, lines 36-43. Andreotti summarized the work of a conventionally known and oft cited patent in the field of ATP extraction and detection, US Patent No. 4,303,752, Kolehmainen et al., 1981 (Exhibit 8, attached hereto). The Kolehmainen patent refers to both ionic surfactants and non-ionic surfactants for use in releasing ATP from cells. Exhibit 8.

15. The term 'microtube' was commonly used by 1995 to refer to a microcentrifuge tube. Exhibits 9 and 10. The depiction of the microtube test unit shown in the drawings of the '081 provisional would have conveyed to one skilled in the art in 1995 the option of using threads as a means of attaching the microtube to a test apparatus. Attached hereto are pages from two standard commercial catalogs: Denville Scientific Inc., *Research Products Catalog 1991,* pages 1, 2, 15 (Exhibit 9) and Denville Scientific Inc., *Research Products Catalog,* 1995, pages 1, 2, 16, (Exhibit 10), both of which were readily available and commonplace before and during 1995. It is clear from page 15 of Exhibit 9 and from page 16 of Exhibit 10 that one skilled in the art would have understood a microtube having the appearance of the microtube in the drawings of the '081 provisional to match the microtubes shown in these catalogs, which are screw cap microtubes. Exhibits 9 and 10.

16. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and

further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Steven J. Saul, Ph.D

Date: 3/25/04

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